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# THE DEVELOPMENT OF BOTANICAL MICROTECHNIQUE

By Gilbert Morgan Smith

## INTRODUCTION

Development of our knowledge of cell structure has been correlated in a large measure with the development of methods of microtechnique, and it is a significant fact that Hooke did not discover the cell until he prepared sections of plant tissues. We are sometimes apt to think of the early investigators as having the same advantages we enjoy, but, in order to properly interpretate the relative value of any early botanical discovery, it is necessary to remember the means available to the worker of that time. In the present article the development of methods of making microscopical preparations will be treated from the botanical standpoint. The history of the microscope, and the development of culture methods will not be considered.

Advances made in microtechnique in Botany and Zoology are closely related. Discoveries made by zoologists in the art of making preparations were adopted by the botanist, while botanical methods were utilized by the zoologist. The microscopists, who were neither botanists nor zoologists, especially those under the influence of the London Society of Microscopists, have also played a very appreciable part in the development of the methods of making microscopical preparations. Probably for their development of microtechnique, rather than for their microscopical discoveries, are we indebted to the greatest extent to the microscopists.

In tracing the development of animal microtechnique Apáthy has divided the time into three grand periods as follows:

1. The rule of the Dry Preparation, which lasted from the discovery of the microscope to the end of the 30's.
2. The rule of the compressorium and the razor, 1840-1880.
3. The rule of the microtome, 1880 to the present.

Perhaps a better method would be the discussion of botanical microtechnique under the following captions:

1. The methods of the early microscopists (from the time of Hooke's discovery of the cell to 1800).
2. The technique of the English microscopists (1800-1875).
3. The methods of the German botanists (1800-1875).
4. The development of modern methods of microtechnique (1875 to the present).

## The Methods of the Early Microscopists

The microscopical laboratory, in the modern sense, did not exist in the 17th and 18th centuries. There are few illustrations of microscopists' work-rooms comparable with those that the chemist, physicist or pharmacist can show us. Ledermuller shows the way that a microscopist of that time fitted up a room for the purpose of showing his friends "some of the wonders of nature" by means of Cuff's solar microscope. An even better illustration of a laboratory is found in the head piece in each volume of Joblot's work (Fig. 1). This is probably an allegorical picture rather than an actual representation, but the microscope before the window, the hand microscope which is being used, the twigs in the vases and various objects on the table suggest the modern laboratory. The conspicuous position of the globe, the telescope and other instruments in the floor, however, do not fit into our present-day concepts but show the catholic taste of the early investigator.

Up to the beginning of the 19th century the microscope was a toy rather than an instrument of scientific research. Nelson mentions Pepys paying £10 for a microscope in 1664 and thinking it "a great price for a curious bauble." The attitude toward microscopy is also shown in the allegorical frontispieces of Ledermuller and of Adams. When Wilson says "In viewing Objects, one ought to be careful not to hinder the light falling on them, by the Hat,

Perruke, or any other Object," we can easily imagine the casual manner in which the gentleman of that time looked through his microscope. Another conception of the purpose of the microscope is that of Baker (1742) who says "And if I can hereby induce any to pass those leisure hours agreeably and usefully, in contemplating the Wonders of Creation, which would otherwise be spent in tiresome Idleness, or, perhaps, some fashionable and expensive Vice, I shall think these Sheets very happily bestowed."

The first microscopists had to make their own microscopes as well as their microscopical preparations and, considering the primitive character of these instruments, it is natural to find them thinking the improvement of the microscope more productive of results than the improvement of the method of making their preparations. During the 18th century almost all works on the microscope were written by microscope manufacturers; so that great emphasis is laid on description of the construction of the instruments. These "Micrographias" were frequently sold with the microscope and were therefore written for the benefit of those who desired to dabble in microscopy. There was little serious use of the microscope, Baker stating the general attitude in the following: "Many, even of those who have purchas'd Microscopes, are so little acquainted with their general and extensive Usefulness, and so much at a Loss for Objects to examine by them; that after diverting themselves and their Friends, some few Times, with what they find in the Sliders bought with them, or two or three more common Things, the Microscopes are laid aside as of little farther Value. . . . ."

Hooke's microscope, as described in the "Micrographia," possessed no stage, the objects being mounted on a point attached to a pedestal at the base. Since Hooke prepared sections when he discovered the cell the following extract of his description is of interest. "I took a good clear piece of cork, and with a pen-knife sharpened as keen as a razor, I cut a piece of it off, and thereby left its surface smooth; then examining it very diligently with the microscope, . . . . but that possibly, if I could use some further diligence, . . . . I, with the same pen-knife, cut off from the former smooth surface an exceedingly thin piece of it: and placing it on a black object-plate, because it was itself a white body, and casting the light

on it with a deep plano-convex glass I could exceedingly plainly see . . . ." A few years later in the Cutlerian Lectures he described a method of fastening "Muscovy Glass" to the bottom of the tube of the microscope in place of the "common Pedestal hitherto made use of in Microscopes." Another method used by Hooke is quite noteworthy, since it was used but little in the century following, and not until about 1820 did the process come into general use. He says: "But there are other substances which none of these ways I have yet mentioned will examine, and those are such parts of animal or vegetable bodies as . . . the Pulps, Piths, Woods, Barks, Leaves, Flowers, etc., of Vegetables . . . but if the same be put into a liquor, as water or very clear Oyl, you may clearly see such a fabrik as is truly very admirable . . . ."

Leeuwenhoek frequently made a microscope for an object that he wished to view and since these objects were generally fixed to a point on the microscope we may consider them as a sort of permanent microscopical preparation. Aside from the allegorical figure holding one of these microscopes in the frontispiece to the "Arcana Naturae" he has left no figures, while descriptions of his microscopes are known only from other writers. Upon his death a cabinet, containing several of these microscopes with their mounts, was left to the Royal Society of London and described by the vice-president, Martin Folks, and by Henry Baker (1753), before they were stolen from the Royal Society. The only original Leeuwenhoek microscope known to be in existence today is in the Utrecht cabinet, the Royal Microscope Society of London having a modern reproduction of the Utrecht microscope. In Folks' description we find; "Mr. Leeuwenhoek, fix'd his Objects, if they were solid to this Silver Point with Glew; and when they were Fluid, or of such a Nature as not to be commodiously view'd unless spread on Glass, he first fitted a little Plate of Talk*, or exceedingly thin-blown Glass, which he afterwards glewed to the needle, in the same manner as his other Objects." The figure of Leeuwenhoek's microscope as given by Ledermuller (Fig. 2) is particularly instructive since it shows the fine needle-like point on which the objects were mounted.

*The old usage of the word talc or "talk" is misleading since it refers to mica and not to the magnesium silicate called talc today.

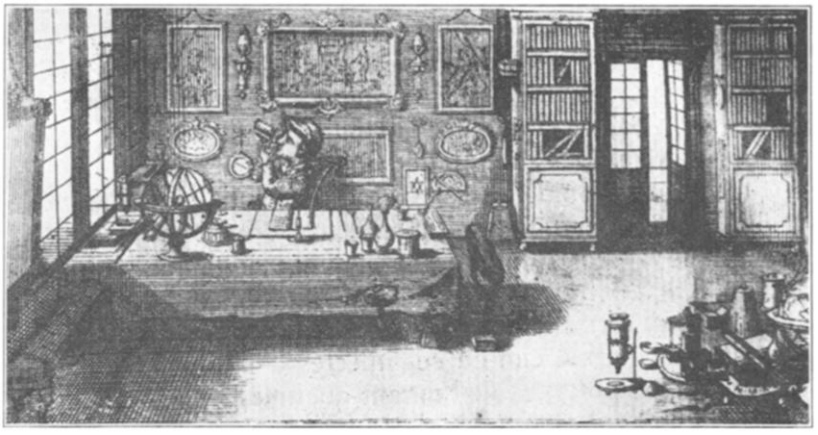


Fig. 1.—Allegorical representation of an eighteenth century microscopist's laboratory. (Joblot, 1754).

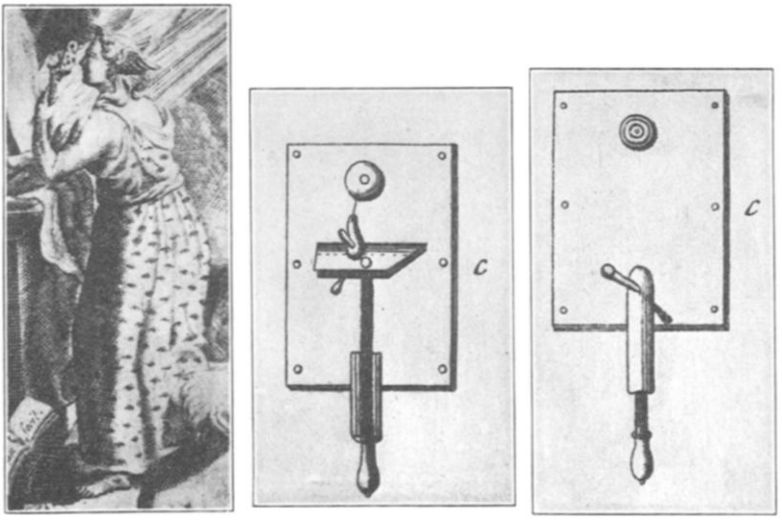



Fig. 2.—A. Leeuwenhoek's microscope (Ledermüller, 1768). B. An allegorical figure showing method of using this microscope. (Leeuwenhoek, 1722).

PLATE I

In his study of blood corpuscles he first made use of small tubes for examining liquids. This method of exmaining material in capillary tubes became obsolete about 1800 but during the 18th century small glass tubes were a part of the equipment of all microscopes and Baker, Martin, and Adams all figure them. Leeuwenhoek's method of preparing these tubes was as follows; (1674) "I did myself prepare divers sorts of very slender hollow Glass pipes, of which some were not thicker than a Mans-hair; . . . . This pipe with the blood in it, I lay upon a piece of white paper, and with my nail break a little piece from it, and set it to the pin of my microscope, having first a little wetted the pin with my spitle, or a little turpentine, to make the pipe stick to it; or else I take the whole Glass-pipe and with my hand hold it before the microscope." This use of glass tubes for mounting material was almost exclusively confined to his zoological studies, and he later made the tubes larger and larger even describing one as large as a finger. (1702).

The founders of plant anatomy, Grew and Malpighi, have left but little record of their working methods. Malpighi says nothing at all about his methods, while all Grew has to say is; "to do all this by several ways of section, oblique, perpendicular, and transverse; all three being requisite, if not to observe, yet the better to Comprehend, some things. And it will be convenient Sometimes to Break, Tear, or otherwise Divide, without a Section. Together with the Knife it will be necessary to joyn the Microscope; and to examine all the Parts."

In the beginning of the 18th century we find object carriers or slides, ("sliders" as they were called at that time) coming into general use. About the earliest record of a slider that we have is the figure of one in position for use in the microscope of Philip Bon-

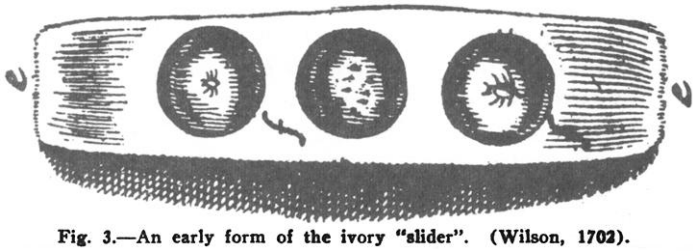


Fig. 3.—An early form of the ivory "slider". (Wilson, 1702).

nani in 1698. Four years later in the Philosophical Transactions, Wilson shows one of these "sliders" (Fig. 3) describing it as follows: "EE a flat *piece of Ivory,* whereof there are 8 belonging to this set of Microscopes, (tho any one who has a mind to keep a Register of Objects may have as many of them as he pleases) in each of *which* there are 3 holes fff, wherein 3 or more Objects are placed between two thin Glasses, or Talks, when to be used with the greater Magnifyers." In this connection it should be borne in mind that material mounted in these sliders was always mounted dry. Hartzoeker (Fig. 4) used a hinged brass frame, in which the material was held between two pieces of mica, a process which was used but little. About the middle of the century glass "sliders" made their



Fig. 4.—Hartzoeker's frame for holding microscopic objects. (Harting, 1859).

appearance; Martin describing "a long piece of Glass, for moving the Object this way and that." The glass slide was not used for permanent preparations Adams (1747), Hill, and "Medicus" mentioning only temporary mounts with the glass "slider."

In 1742 Henry Baker devoted a chapter of seven pages to the subject "of preparing and applying objects." The need of preparation is seen in his statement that "Most Objects require some Management, in order to bring them properly before the Glass." The first method described, that of dry mounting in the "slider," is recommended for use wherever possible. Small concave glasses, quite similar to the watch crystals now in general use, are suggested for examining fluids containing organisms. In mounting these the material is to be taken up by means of a brush, which is figured among the microscopical accessories (Fig. 5 A). Baker also suggests the use of slips of glass, the same size as sliders, so that objects could be placed on them for examination; the interesting feature being the recommending glasses of different colors, giving as his reason; "many Objects being much more distinguishable when placed on one Color than on another." "Opake" objects are to be placed on small slips of colored cardboard, about half an inch in

length and a tenth of an inch in width, and then fastened to the cardboard with mucilage. For preserving these preparations Baker

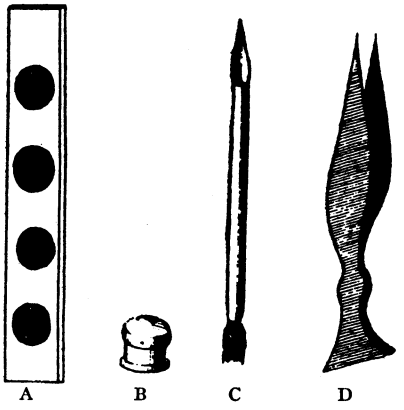



Fig. 5.—Apparatus for making microscopical preparations. A. Ivory Slider. B. Box of talcs. C. Camel's hair brush. D. Brass nippers. (Baker, 1742).

devised a box fitted up with compartments (Fig. 6), this being the first record that we have of boxes for keeping preparations.

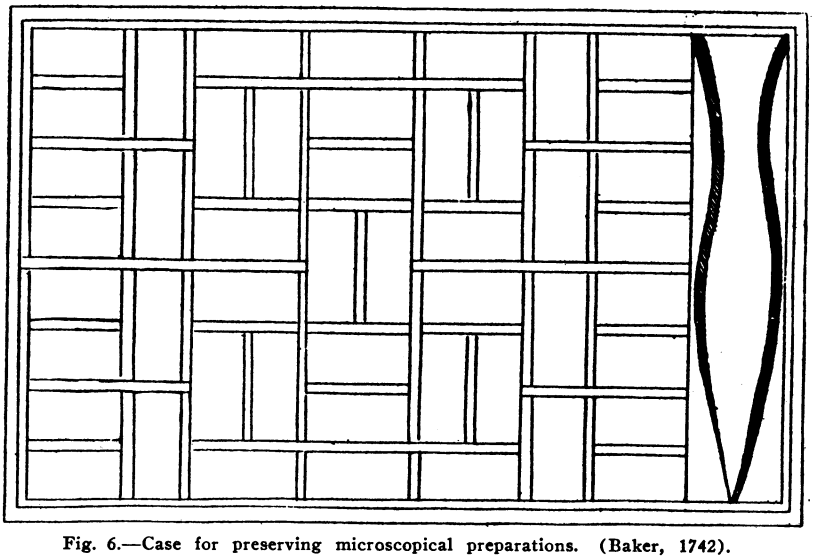


Fig. 6.—Case for preserving microscopical preparations. (Baker, 1742).

Pollen grains are among the microscopic objects recommended by Baker and in Chapter 22 is found the method of making preparations of "Farina" (the old name for pollen grains). "Gather your *Farina* in the midst of a Sunshiny dry Day, when the Dew is off; be careful not to squeeze or press it, but shake or else gently brush it off with a soft Hair pencil upon a piece of clean white Paper. Then take a single Talc or Isinglass between your nippers, and breathing on it, apply it instantly to the *Farina,* which the moisture of your Breath will make adhere to it. If too great a quantity of Powder seems sticking to your Isinglass, gently blow off a little; if there be not enough breath on it again, and touch the *Farina* with it as before. Then put your Glass into the Hole of a Slider, and apply it to the *Microscope* to see if the little Grains are spread according to your liking, and when you find they are, cover them cautiously with another Talc, which fasten down with brass Wire, but let not the Glasses press hard upon the *Farina,* for that will destroy its true Figure, and represent it different from what it is."

Two further contributions to microtechnique which appeared in this century are in use to the present day. Ledermuller devised the use of dipping rods for removing material from a liquid (Fig. 7); while Benjamin Martin gives the first hint of maceration when he says; "If *Leaves* are steep'd in Water for Maceration, the Pellicle or thin Skin of both Sides will easily peel off, which laid on a glass and view'd with the Light reflected thro them, will discover a most delicate Texture. . . . ."

Historians emphasize the barrenness of the 18th century, as compared with the 17th, in the development of the microscope. With one notable exception, this is also true for microtechnique. The great originality which John Hill showed in the manipulation of the material described in his work entitled "The construction of timber, explained by the microscope" has not been given due credit. In his biography of John Hill, T. G. Hill has entirely failed to call attention to one of the most important features of John Hill's work, namely, the superiority of the microtechnique which he employed. Methods were used which had not been employed up to that time and which did not come into general use until fifty years later, and then as rediscoveries by others.

Hill did not rely on any one method but studied the structure of stems in many different ways. He used a more elaborate method of maceration than Martin, and was the first to use maceration in the study of wood, sinking a loose wicker basket containing the sticks he wished to study into a stream until the tissues were well

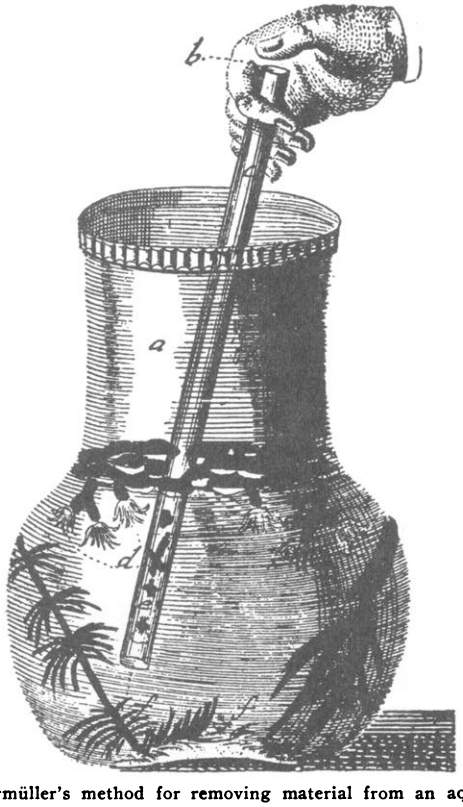



Fig. 7.—Ledermüller's method for removing material from an aquarium. (1768).

softened. This identical method which was rediscovered by the younger Moldenhawer in 1812 is considered by Sachs as one of the great steps in the progress of phytotomy. Hill also makes the first mention of a method of preservation of material for further study. The practice of dropping the macerated pieces of wood into a solution of alum and then transfering them to spirits of wine, after

thoroughly drying, resembles in a very crude manner our modern method of fixing and hardening. The reason for this is seen in his statement that "Nothing but spirit of wine can preserve these tender bodies, and, till I found this method of hardening them first, the liquor often destroyed them."

Holzner thinks that Sarrabat or Reichel should have the credit for being the first to use staining methods since they put sticks into colored liquids and then noted the rise of the color. Apáthy has raised the question as to whether this work should be regarded as at all comparable to our modern methods of staining microscopical preparations. Judging by the excerpts cited from these articles by Holzner they were macroscopical studies only, and it is very probable that Hill was not aware of them, or the work of Bonnet.

Hill is undoubtedly the first to have used staining as an aid in the study of microscopical anatomy of plants. He prepared an alcoholic tincture ofcochineal, in which, after it had been filtered, he placed the stems of plants for a while, discarding that portion which had been immersed in the fluid when he made his sections. Another method of staining used was even more advanced since it involved a mordanting of the tissues before developing the color. A solution of sugar of lead was prepared, filtered, and put in a tea-cup and the sticks to be studied were allowed to remain in this fluid for two days. An essential part of this operation was the "whelming" of the tea-cup with a wine glass to prevent the drying of the material. While the tissue was soaking in the lead solution he prepared a solution of quick lime and orpiment in water and then transferred the material from the tea-cup to the second solution for two days. When the sticks were first placed in the second solution they were colorless, but in a short time they became deep brown. By means of this staining he was able to demonstrate the existence of structures invinsible in the uncolored material. A third method, which was an injection rather than a staining process, was the careful drying of the wood and then boiling it in green sealing wax. By this procedure the vessels became thoroughly impregnated with the green sealing wax and the "split pieces resemble striped satins, in a way scarce to be credited."

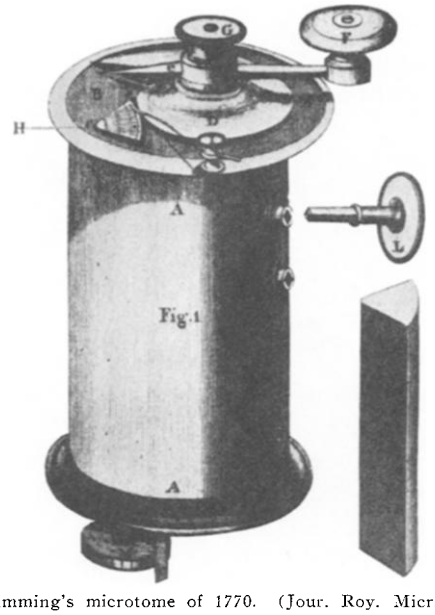



Fig. 8.—Cumming's microtome of 1770. (Jour. Roy. Micr. Soc., 1910).

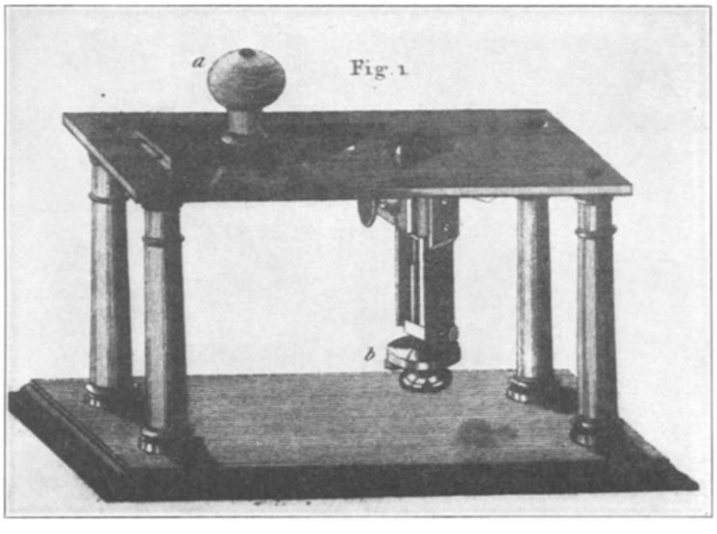



Fig. 9.—Adam's cutting engine. (1798).

PLATE II

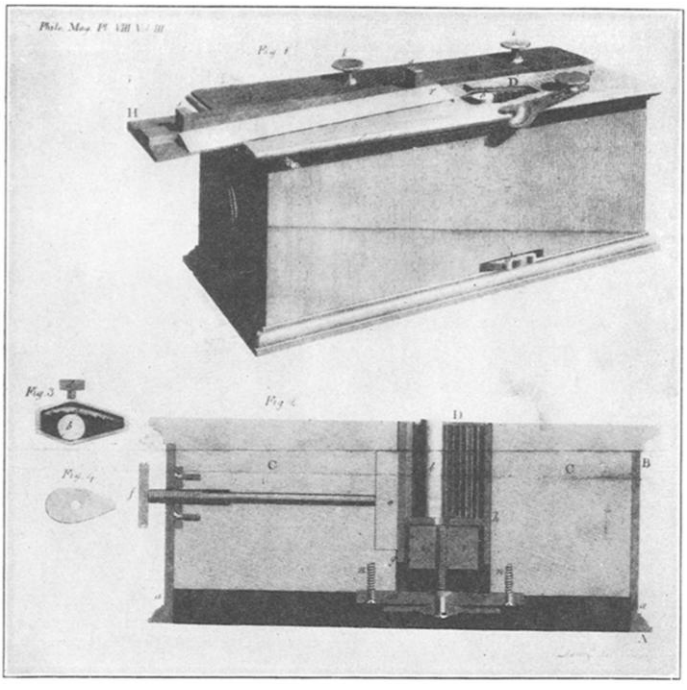


Fig. 10.—Custace's cutting engine. (Thornton, 1799).

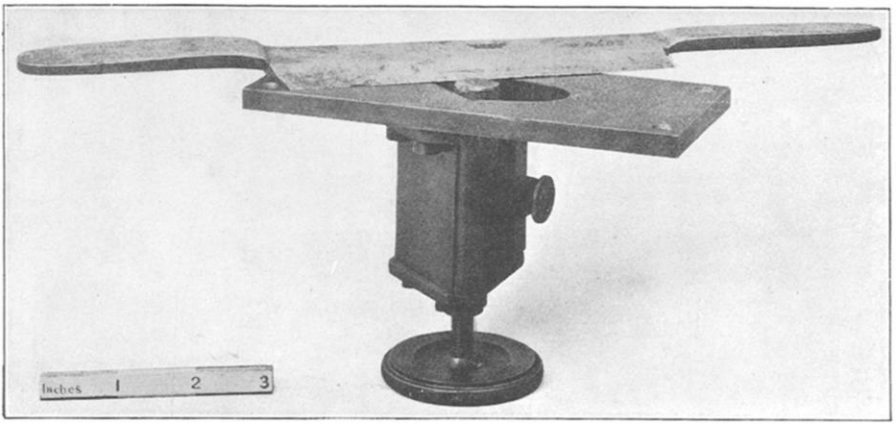



Fig. 12.—Pritchard's microtome (1835).

PLATE III

Other methods of study used by Hill include the placing of tissues of pine in spirits of wine until the resins were dissolved out and the cells rendered more visible. Another, the placing of tissues in spirits of turpentine until the contents became clear. Both of these methods, although extremely valuable in the study of the anatomy of plants were not used until a much later period. In this study a slide of ground glass was used in addition to the ivory "slider", but both of them were used in the holder devised for the purpose instead of placing them directly on the stage of the microscope. As to his methods of making mounts with the glass he says: "it is to be examined, if fresh, in water; if preserved in some of the spirits in which it is kept; being laid in a little cistern hollowed in a slip of ground glass." This form of slide devised by Hill is generally regarded as being a comparatively modern invention. These methods of study developed by Hill received but little notice, Adams seemingly being one of the few to recognize their value as is seen when we read; "it were to be wished a satisfactory account could here be given of all the preparations which are requisite to fit for the microscope the objects of the vegetable kingdom. Dr. Hill is the only writer who has handled this subject."

The sections that Hill used were cut out on a microtome. Queckett states that the first cutting machine (microtome in our sense) was made by Adams about 1770. The instrument that Hill used (Fig. 8) was one invented by Cummings and was probably well known at the time since after making two or three Cummings turned their manufacture over to Ramsden who supplied them to those desiring "cutting engines." The body of the instrument (AA) was made of ivory, while the top was of bell metal. The spiral-edged cutter was so arranged that the difference between the longest and shortest radii was greater than the thickness of the largest piece of wood that the instrument would hold. The handle (F) was used to revolve the spiral cutting blade and after each revolution of the blade the material being sectioned (H) was raised the desired height by means of a screw (M), each division on the head of the screw corresponding to an elevation of the material 1-1000 of an inch. Hill was troubled by the sections of wood curling as they were cut, so a fine spring was used to keep them flat. After

cutting, the sections were transferred to spirits of wine. Those interested in a more complete description than is given here, and to whom the work of Hill is inaccessible, will find a reprint of Hill's description and plate in the Journal of the Royal Society for 1910.

Thanks to the love of the early microscopists for careful descriptions of the minutiae of manipulation we have a good account of the state of microtechnique at the close of the 18th century given by Adams. In these "Essays on the Microscope" a description is given of all the microscopes in use at that time, and, what is of greater interest from our point of view, he carefully figures and describes all the accessory apparatus which accompanied those microscopes. All were supplied with ivory sliders which fitted into slider holders made of brass, while the objects were mounted dry between pieces of talc and held in the holes of the sliders by brass wires (Fig. 5 A). The sliders as supplied usually contained objects ready for examination, but an empty slider or so was sent along, as well as a box of extra talcs (Fig. 5 B), so that the owner of the microscope could make preparations if he desired. Other accessories needed in the preparation of objects were camel's hair brushes (Fig. 5 C) and brass nippers for adjusting the brass rings that held the talcs in place (Fig. 5 D). Approximately half a century separate the first edition of Baker's "The microscope made easy" and the last edition of Adams' "Essays" yet little improvement is noted in the accessory apparatus figured, the apparent advancement shown in a comparison of the figures of Adams and Baker being due rather to better draughtmanship.

Adams described an improved instrument "for cutting thin transverse sections of wood." This "cutting engine" (Fig 9) consisted of a wooden base which supported four brass pillars that in turn bore a flat plate of brass, in the center of which was a triangular hole. The piece being sectioned was placed in a triangular trough on the under side of the brass plate, and fastened by a brass screw. A diagonal knife blade, greatly resembling the blade of a plane, did the cutting. This was moved back and forth by a handle, its course being governed by two grooves in the top of the brass plate. The amount that the block was raised was governed by

a micrometer screw. In cutting, only fresh material or well soaked material was used and this was kept flooded with alcohol to prevent the curling of the sections. That other microtomes besides those of Cummings, Adams, and Custace were known may be judged by the foot-note of Kanmacher (the editor of the second edition of Adams' "Essays"). "Many other kinds of cutting engines have been constructed, but specimens from them have not yet appeared with the perfection which is requisite to this sort of objects; whether it lies in the preparations of the woods, or engine, I do not take on me to determine."

In this work also appears the first record of the dealer in microscopical preparations, the last chapter containing a list of "vegetable cuttings" which Custace supplied to those interested in microscopy. His sections were all prepared by means of the microtome that Thornton has described. Custace was quite famous for the sections that he made, his preparations being supplied with all the high-grade microscopes of that time. Even as late as 1852 we find Queckett saying: "some of his (Custace) preparations have not been improved on to the present day." Custace, who was a "common carpenter of Ipswich" kept his methods of making preparations secret during his life, refusing an offer of £50 from Thornton for a description of his methods. After his death all his effects were auctioned off and Thornton "fearful that a monopoly might be made of the art of preparing vegetable cuttings, as had been successfully done by Custace," bid in the two microtomes offered for sale. Thanks to the generosity of Thornton we have a description of these "cutting engines." The outer case of the cutting machine (Fig. 10) was made of brass, in the form of an oblong box, which was completely filled with a block of hard mahogany except for the holes necessary for the mechanism that held and raised the material being sectioned. The block was raised by a micrometer screw, which is not shown in the illustration, the screw being operated by an index wheel (O) at the side of the box. The large screw at the left of the microtome was used to clamp the block in the brass "Holdfast" (D). Especial attention was paid to the designing of the mechanical means for guiding the knife, and it may be due to this that the machine cut such good sections. The knife blade was

set diagonally on a bed (H) which slid back and forth along a steel rod (GB). Another device for securing rigidity was the placing of a spur (u) on the top of the microtome. This spur held the material in its crotch and was prevented from giving by a screw (v).

In concluding the discussion of microtechnique before 1800 the following items might be of interest. They are taken from the advertisement of W. & S. Jones and appeared in Adams' "Essays."

| | £ | s | d |
|---|---|---|---|
| Common Microscopes from 2 s. 6 d to..... | 1 | 1 | 0 |
| Compound Microscopes from 2/12/2 to..... | 5 | 5 | 0 |
| New Improved ditto with most complete apparatus.... | 10 | 10 | 0 |
| Cutting Engine for slices of vegetable objects..... | 3 | 3 | 0 |
| Ivory Sliders, for transparent objects (per doz.)..... | 0 | 12 | 0 |
| Custace fine vegetable cuttings in large ivory sliders, from a set of six sliders to four dozen (per doz.).... | 1 | 10 | 0 |

## The Technique of the English Microscopists (1800-1875)

With the beginning of the 19th century we find botanical microtechnique developing along two distinct lines. With the rise of the German phytotomists there was a high degree of specialization along the line of microchemistry while comparatively little attention was paid to refinements in methods of making preparations. In England, on the other hand, with the reawakening of interest in the microscope, attention was largely confined to developing methods of making microscopic preparations. Non-scientific Englishmen, as well as the microscopists, were interested in the microscope and so we frequently find articles on the microscope, or on microscopic objects in the popular magazines. Examples of these popular articles on the microscope are those which appeared in the *Saturday Magazine* or the *Mirror*. Popular treatises on the microscope also appeared and through the influence of such works as those of Brewster or Pritchard (1847) there was a recruiting of a body of microscopists. With a few notable exceptions the English microscopist made no great contributions to botany, that were based on microscopical observation, but they were largely instrumental in advancing microtechnique. It has been through the refinements zoologists have made in the Eiglish microscopists' methods, and then in turn their adaptation by botanists that we have the botanical microtechnique of today. Indirectly also we are indebted to the microscopists

for the great stimulus that their interest in the microscope, and their willingness to purchase it, gave toward the improvement of the instrument; hence the development of the microscope up to comparatively recently has been due almost wholly to demands of the microscopist for a better instrument.

I have previously mentioned that the early microscopists did not work in regular laboratories. A great majority of the men in England who were interested in microscopy in the period under discussion were not connected with an institution where they would have regular work-rooms, so worked in their homes. Consequently writers on microscopy give directions for the best conditions for work. Goring and Pritchard recommend a separate room, which they call an observatory, that is always to be kept locked when not in use. The difficulties with which these workers were beset is brought out in the following: "Have the fear of the cat before your eyes, and also those busy, intermeddling, officious, housewives, who, under pretense of dusting, cleaning, and setting to rights, will subvert and revolutionize the whole economy of your observatory, and perhaps throw half your tackle behind the fire."

The use of the ivory and wooden slider continued until the first or second decades of the 19th century, though they did not become entirely obsolete until about 1860, some of the inferior microscopes at that time being furnished with them. With the general introduction of the glass slide the methods devised for the ivory slider were adapted to the glass slider with a few changes. This modified method is described by Gould who in making permanent preparations took two pieces of glass, of the same size, and then pasted on one a piece of paper containing one or two holes. After the mucilage was dry the material was placed in the holes in the paper, the other glass pasted on the paper, and the whole held together until thoroughly dry. That this process became quite general is seen in its description by Griffith, von Mohl, Queckett, Harting, and others.

The publications appearing in the years 1830-1835 mark the foundation of the English microscopist's methods. It is impossible to state who first substituted the glass slide with a cover of talc for the ivory or wooden slider in making preparations, but that it was a well

known method by 1830 may be judged by its use by Pritchard (1832) Varley, Solly and Holland. Pritchard's revolutionary method of mounting dissections in a thick solution of gum and isinglass and then covering them with a thin plate of talc was described in 1832 but did not come into general use since it was so soon superseded by mounting in Canada balsam. Credit for the introduction of Canada Balsam is generally given to J. T. Cooper who suggested its use to New and Bond, professional microtechnicians of that time, but the publication of the method is due to Pritchard (1835). Balsam may have been used before this since Adams states the following about Swammerdam's methods of preparing insects for microscopical observation: "Sometimes he has examined with the greatest success, and made the most important discoveries in insects he had preserved in balsam, and kept for years together in that condition." I have been unable to find this description in the work of Swammerdam, but Adams may possibly have taken this from Boerhaave, an article which I have not personally consulted. However, it was through Pritchard's publication that Canada balsam became widely known as a mounting medium. The first mounts made differed considerably from those we now use since Canada balsam was taken in the natural state, instead of dissolving it in some solvent, and after melting a small piece on a slide the object was mounted before the balsam hardened. Judging by the space given to the description of the process in the older works on microscopy there must have been considerable difficulty in making the preparations and all sorts of mechanical contrivances were devised to hold the cover in place during the drying of the balsam, to melt the balsam, to remove air bubbles from in under the cover glass, etc.

The development of methods of mounting objects in liquids is of even greater significance. The earliest record I have been able to find is by Goring and is as follows: "I have neglected to describe a kind of slider which I use in my microscope; it is composed of a glass tube, flattened, and drawn out to the size of a common slider, and polished on one side; its use is to hold microscopic objects which will not keep in a dry state, such as pieces of finely injected membrane, petals of flowers, and the like; these little preparations are introduced into the slider, which is filled with spirits, and cov-

ered at the end with a bit of bladder secured by a wax thread". This is the only record of this type of permanent preparation. In 1829 a short anonymous note stated that Holland had covered material, which showed the Brownian movement, with a talc and then hermetically sealed the preparation. Four years later Holland described the method of making these "ponds" by taking a piece of glass and enclosing a space on the glass with a cement composed of white lead and turpentine and then covering with a talc and sealing. Although devised to show the Brownian movement Holland stated that the "pond" could be used for tissues. Previous to the publication of the process by Holland, Varley had described essentially the same method for preserving "minute vegetable dissections" and called this type of preparation a "cell," the name by which they are universally known at the present day. White lead cells were also made as early as 1830 by Valentine, Solly mentioning preparations of Valentine's made at that date. It is possible that this type of preparation was known even earlier for in 1841 Daniel Cooper said that Dr. Cook recommended a mixture of salt and water as a mounting fluid "20 years ago" (i. e. about 1820). Pritchard's process (1832) is even more important for he is the first to have suggested mercury bichlorid as a mounting fluid. Daniel Cooper says that J. T. Cooper used salt and water with a little acetic acid for mounting vegetable tissues, although he does not give the date on which Cooper proposed this method.

Goadby is generally credited with being the founder of the methods of making moist preparations, but the citations just given show that methods of making moist preparations were well known before the work of Goadby and any credit due him is more for perfecting methods in use than for original discovery. He is best known for the mounting fluid that bears his name. That this process was recognized as being revolutionary is seen by the fact that the Society of Arts gave him a gold medal in 1841 and raised a private subscription of £500 to purchase his preparations for the Hunterian Collection of the Royal College of Surgeons (Goadby, 1852). The formula for the solution was first published by Daniel Cooper in 1841, but since there seems to have been considerable difficulty in making preparations in this way Goadby described his meth-

ods and gave his formulae before the Section of Botany at the York meeting of the British Association in 1844. The Gannel process described by Cooper is unknown except for this single reference. It was quite similar to Goadby's method except that "superacetate of aluminia" was used.

The attitude of the British microscopists towards their discoveries was in marked contrast to that of investigators of other countries. There seems to have been a great freedom of oral interchange of ideas, and one man often devised a really epoch-making improvement in microtechnique but made no attempt to publish it, being content to communicate his discovery to his friends by word of mouth. Michael has explained how microscopists came to see so much of one another when he says that Bowerbank practically kept open house for microscopists before the formation of the Microscopical Society of London. After the formation of the Society in 1839 there was a formal meeting place for microscopists but the practice of holding "soirees" at various times favored the oral interchange of ideas. To mention only a few instances, we have Pritchard publishing Cooper's method of mounting in Canada balsam, Cooper publishing Goadby's formulæ, and Clarke the first method of clearing, a process which he may possibly not have invented.

Holland is the first to describe the deep cell that is made by applying successfully several layers of cement. The importance of the cement cell in microscopy at this time is seen in the number of

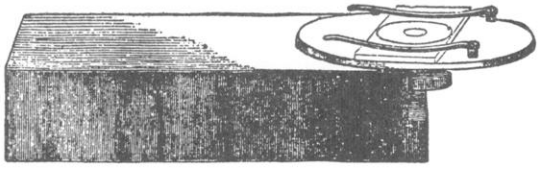


Fig. 11.—An early form of turn-table for making cement cells. (Carpenter, 1856).

cements described by Griffith and Henfry, Queckett, Carpenter, and Beale. The chief contributions to the subject of cements were Berkley's description of Thwaite's method of using gold size, and Reckitt's recommendation of black Japan on account of its quick drying properties. At first these cells were made by hand, but the invention

of the turn-table by Shadbolt gave a quick mechanical means of making them. The first turn-table is figured by Queckett, but it was soon improved and the instrument we use today shows little advance over the form given by Carpenter in 1857 (Fig. 11).

Griffith gives the status of microtechnique in the early forties where he says that the use of ivory and wooden sliders has almost disappeared but he describes a method of dry mounting very similar to that of Gould. The method of mounting dry objects in balsam, and several methods of making moist preparations are also given. For the latter a syrup and gum mixture, dilute alcohol, water saturated with creosote, and Goadby's solution are described, the last named being given the preference. In mounting these preparations the sealing with some varnish, the use of white lead cells, and built-up cells are all described.

Goadby's solution proved altogether too strong for plant material and consequently a number of media were devised which had as their object the avoidance of plasmolysis. Thus Reckitt advised sealing the tissues in either pure water or a dilute solution of corrosive sublimate, while in 1849 Warrington recommended castor oil for certain fungi since he had found this such a good mounting medium for crystals a few years previous. The desmids have always been a favorite object for study with the microscopists and we therefore very naturally find several formulæ of mounting fluids for these delicate organisms; among them may be cited Thwaites' mixture of 1 part alcohol and 12 parts water with as much creosote as could be dissolved, a proportion which was later changed to 1 part alcohol to 16 parts of water when the process was described in Ralfs' work on the desmids. Ralfs medium for desmids consisted of a grain each of bay salt and alum dissolved in an ounce of water. Glycerine was first used by Warrington in 1849 for mounting microscopical preparations, but since glycerine alone caused too much plasmolysis Farrants proposed a jelly of equal parts of glycerine, gum arabic and water, while a formula essentially the same as the glycerine jelly used today was given by Lawrance in 1859. The idea of using gelatine as the foundation for the mounting fluid really belongs to Deane who first proposed a mixture of honey, water, alcohol, creosote, and gelatine.

Although all of these methods were described before 1860, we must bear in mind that they were used only in special cases, Canada balsam being considered the prëeminent medium whenever possible. The column of questions and answers appearing in *Science Gossip* during the sixties contains many more references to Canada balsam than to any other method. A revolutionary step in the manipulation of Canada balsam was the dissolving of the balsam before mounting. This was first used by Griffith in 1843 but does not appear in the article on microtechnique by Griffith but as an editorial note of a few lines in the same volume that contains his description of microtechnique. The use of a solvent for the balsam did not come into general use in England, until more than a decade later about the only reference to it being those of Boys and Ralph.

Another great step in microtechnique appearing in the decade between 1850 and 1860 is the introduction of the process of clearing tissue before mounting them. In 1851 Clarke, in describing his treatment of certain animal tissues, stated that he put them in spirits of wine, then transferred them to turpentine and after they had become quite clear mounted in balsam. It is possible that he did not originate this method since Farrants remarked in 1857, incidental to a discussion of the use of glycerine, that he had cleared his material in turpentine before mounting in balsam since 1850, but makes no mention of Clarke's name. From the historical standpoint Clarke's publication of the process is important for it was through his description that it became known abroad and in the hands of the German zoologists of 1860-1870 developed into the methods of clearing that still persist. Evidently the value of this method was not recognized in England since the treatises of Queckett, Griffith and Henfry, Carpenter and Beale all fail to mention it.

The cutting engine as invented by Adams, Cummings, and Custace was used more or less in England between 1800 and 1870. After speaking about these 18th century microtomes Queckett goes on to say "in subsequent times other instruments have been contrived for the same purpose, some provided with knives that move circularly, others with knives fixed in a strong framework of metal, whilst, in not a few, the cutting is performed by a razor of the ordinary kind, or one ground perfectly flat." I have been able to find

reference to five microtomes, other than the two described in the first edition of Queckett in 1848, that were made before 1850 but judging by the statement of Queckett many more were known. Michael says that before the foundation of the Microscopical Society of London George Jackson made "a very servicable cutting-machine for producing thin sections of wood." The firm of Charles Baker of London inform me by letter that they have been making microtomes since about 1840. In 1836 Bowerbank described a microtome that he had invented which was quite similar to the Adams instrument, the chief difference being that the cutting part of the instrument was a razor ground flat on one side. There are also two old microtomes in the Science Museum, South Kensington, London, that were made about 1835*, one bearing the name of Andrew Pritchard, while the other bears no maker's name but is of very similar design. Pritchard's microtome (Fig. 12) is described as follows in the Science Museum catalogue. "The apparatus is made to be screwed to the edge of a table and consists of a flat plate of brass with a well in it, in which a kind of piston moves up and down by a micrometer screw. The wood to be cut is fixed to the piston by a small screw, and as it is raised a knife drawn along the plane surface takes off thin sections. Should the piece of wood be too small to be placed in the triangular chamber, it must be glued to a block of convenient size." This is the first record of a microtome fixed to a table and is the forerunner of the hand microtome.

The instrument of Topping was patterned after the Pritchard microtome and was the best known microtome of the 50's, being figured and described by Queckett, Carpenter, and Beale. It is quite similar to the hand microtomes of the present day, resembling them more than the Pritchard microtome. Another microtome is one that is ascribed to Queckett by Harting and Apathy, although I find no direct evidence that Queckett invented this instrument. It consisted of a mahogany base (Fig. 13) that supported four brass pillars and a top plate. The "well" for holding the material was essentially the same as has been previously described for the Adams cutting engine.

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* I am under very great obligations to Dr. A. B. Bendle of the British Museum for his kindness in furnishing me information concerning these microtomes, and to Capt. H. G. Lyons for the photograph and description of the Pritchard microtome.

The knife differs considerably from that of the Adams cutting engine in that it was placed diagonally on a brass frame, the whole sliding backwards and forwards along a guide rod on the top of the cutting engine.

The great interest in microscopy in the decade of 1850-1860 is evinced by the appearance of five treatises on the microscope and microscopic manipulation. With the appearance of these works, methods which had been but little known outside of a small circle of

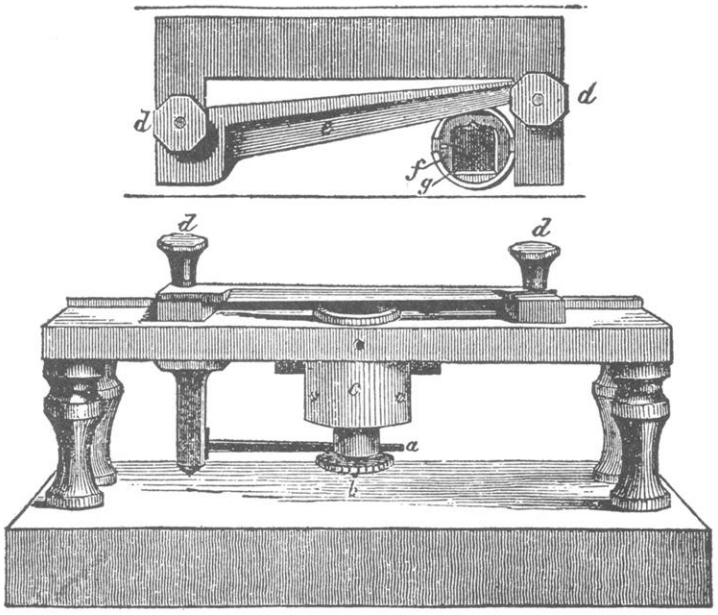



Fig. 13.—Queckett's (?) microtome. (Queckett, 1848).

microscopists became the property of the world and were taken up and perfected outside of England. The advances made in microtechnique by the introduction of the complicated paraffine method made the science one for the laboratory rather than for the home and although interest in microscopy continued in England the progress of the science took place in laboratories of the German zoologists. The most important contribution of the microscopists to microtechnique between 1860 and 1875 was the introduction of staining,

particularly the staining of plant tissues, although the German botanists did not avail themselves of the staining methods of the English microscopists. Since the discussion of this subject will be taken up later no further mention will be made here.

The French microscopists were dominated to a large extent by the English school of microscopists and have played a relatively unimportant rôle in the development of microtechnique. Several of the English works on the microscope were translated into French, Lebour's Galerie Microscopique, for example, being the French edition of Pritchard's Microscopic Cabinet. In the works written by the French themselves English methods were drawn on to a much greater extent than the German, Chevalier and Dujardin both showing quite strongly the influence of the English microscopists. Chevalier deserves credit for being one of the first to substitute thin glass covers for the talc in making permanent preparations.

## Botanical Microtechnique in Germany 1800-1875

As far as the art of making microscopical preparations is concerned the technique of the German botanists was far behind that which the English microscopists used in making preparations of botanical material during the period described above. This was due to the German botanist's belief in the utmost simplicity. To emphasize the necessity of great dexterity with a few instruments, von Mohl, Harting, and Behrens all quote Benjamin Franklin's adage that "a naturalist must saw with an auger and bore with a saw". Such great manual dexterity was developed in cutting free-hand sections, that mechanical instruments were thought to be only for those who could not make good free-hand sections. Thus in his review of the description of the Oschatz microtome, von Mohl held that the Oschatz microtome was of real value only when one wished to prepare large sections for a microscopic cabinet, and that for scientific investigation the microtome was highly superfluous.

In Germany as in England the dry mount was used exclusively at first. With the rise of the Phytotomists there is some evidence of use of water mounts, although it is not clear whether the material used by Link and Kaulfuss was merely examined in a drop of water or whether this drop was covered as we do today. The water

mount was quite generally used by the later Phytotomists; Meyen in his directions for manipulating the microscope (1830) telling us to use a glass slide and a drop of water and then cover it. No permanent preparations were made in Germany before 1840, all sections made with the razor being examined in temporary water mounts.

Kaiser (1877) states that Germany did not take up microscopy until about 1839 and that Moser was the first to bring about a development of microtechnique, while Behrens thinks that von Mohl was largely instrumental in creating this interest in microtechnique. The chief contributions to the technique of making permanent preparations are those of von Mohl, Oschatz, Schact, and Harting. English methods were not drawn on until about 1850 although the papers of Griffith and Varley had been translated into German. The year of the translation of Griffith's paper marks the appearance of Oschatz' methods of making permanent preparations. Oschatz being a microscopist, discussed both animal and plant tissues. For the latter water alone was not recommended but either a concentrated sugar solution, or a sugar solution containing a little acetic acid was recommended. When the young plant tissues were too opaque, Oschatz found that they could be cleared to a considerable extent by placing them in acteic acid before mounting. The following year Moleschott described Harting's process of mounting plant material in a concentrated solution of iron-free calcium chlorid so that the technique of making permanent preparations received a great impetus at this time. Harting took a slide and pasted a strip of paper on each end and then mounted the material in a drop of the calcium chlorid solution in the center of the slide. This was covered with another slide of the same size and the two fastened together with paste on the strip of paper. There was no necessity for sealing this preparation since the hygroscopic nature of the calcium chlorid prevented evaporation. An interesting side light showing what was considered essential in the study of the cell at that time is found in Moleschott's comment on the availability of the method. He says that apart from the swelling and dissolution of the starch grains, the dissolution of the nucleus in a few months, and the shrinkage of the surrounding membrane in many cases (von Mohl's

primordial utricle), the method of mounting in calcium chlorid solution is a very good one. Immediately following Moleschott's description there is a comparison by von Mohl of Oschatz' and Harting's methods in which he thinks Harting's process is the superior and he bemoans the fact that it had not been discovered earlier. The chief objection to mounting in a sugar solution was the inability to seal the preparation properly, von Mohl's preparations usually not lasting over a year. On the other hand Münter thought Oschatz' method the best. The "Mikrographie" of von Mohl is one of the first collections of its kind in Germany and gives a good idea of the methods in use at that time. It may be well to note that the first edition of Queckett, which appeared at approximately the same date, devotes about half the pages to methods of microtechnique, while in the 277 pages of von Mohl's work only 27 pages are given to the subject, thus showing the difference between what was considered essential in microscopy in England and in Germany. Von Mohl thought that most organic bodies should be studied in water mounts since balsam rendered them too transparent. A method of mounting dry preparations, essentially like that of Gould, was described. The technique of Oschatz, Griffith, Thwaites and Reckitt was described but scarcely any attention paid to the making of cells, all preparations containing fluids being hermetically sealed by some varnish after the cover had been placed in position. The calcium chlorid method of Harting was recommended whenever possible, but the swelling of the starch grains and the shrinking of the primordial utricle prevented its universal use. Owing to his strong advocacy of calcium chlorid von Mohl is frequently credited with being the originator of this method.

The formation of the "Verein für Mikroskopie zu Giessen" in 1856 helped standardize methods. This society adopted, after considerable experimentation, a uniform object carrier for all those members who wished to exchange preparations. They rejected the English size of 1 by 3 inches, a form that came into use in that country soon after the foundation of the Microscopical Society of Lon-

don, and used one 33 by 28 mm. instead.* In the by-laws of the society reported by Leuckart and Welcker the following abbreviations show the mounting materials most generally used in Germany in 1856; Al. alcohol, CB Canada balsam, CC calcium chloride, Gi. gum arabic, Gl. glycerine, Lc. liq. conservatoire (Paccini's fluid), WG water glass, Z. sugar, O. dry mount.

The German botanists were familiar with the publications of the English microscopists which were appearing about this time and so we find a gradual abandonment of the calcium chlorid and the concentrated sugar solutions as the exclusive mounting media. This recognition of the English microscopists' methods seems to have been due to Welcker's publication, judging by the statement of von Mohl in 1857. Unfortunately I have been unable to consult the original paper of Welcker. Von Mohl here gives the preference to glycerine over calcium chlorid as a mounting medium. Another publication which greatly influenced botanical microtechnique was that of Schact, which appeared in 1851. This is the first work devoted exclusively to plant histology. Comparatively little attention is given to making permanent preparations, only three methods being mentioned, namely, the use of calcium chlorid, sweet oil, and Canada balsam, but because of the minute directions for the anatomical study of different plants the book was of great value. This work also illustrates the difference between the microscopical methods of the English and the Germans. In the case of the English the preparation was the main thing, while with the Germans the preparation was only a means to an end.

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*The proper size for the object carrier was a subject of considerable controversy in Germany. The following are some of the sizes recommended.

78 x 26 mm. (London format, 1840)
70 x 22 mm. (von Mohl, 1840)
30 x 40 mm. Diameter circular plates. (von Mohl, 1840)
2/3 x 2/3 in. (Oschatz, 1851)
33 x 28 mm. (Giessen format, 1856)
55 x 26 mm. (Frankfort format, cited by Leuckart and Welcker in 1857)
70 x 20 mm. (Gerlach, cited by Leuckert and Welcker in 1857)
37 x 22 mm. (von Mohl, 1857)
43 x 28 mm. (von Mohl, 1857)
48 x 28 mm. (New Giessen format, date of introduction unknown)
65 x 25 mm. (Vienna format)

Perhaps the new Giessen format of 48 x 28 mm. has been the most used in Germany up to the last decade, but at present the English format is in almost universal use. For other sizes less frequently used in modern times see Behrens.

If the German botanist was not fully abreast of developments in the technique of making preparations, he more than compensated for this in the forwarding of botanical microchemistry. By 1800 unorganized studies are found in which nitric acid, hydrochloric acid, the solvent action of alcohol, and the like were used in an attempt to discover the nature of the cell contents. Perhaps the researches of the French botanist, Girod-Chantrans may be taken as typical of this blind groping toward a microchemical study of the plant tissues. One of the earliest definite microchemical reactions is the discovery by Link in 1807 in which he used iron sulphate for determining tannic acid in leaves. In the same article Link relied on warm water, sulphuric and nitric acids as a test for starch. The macrochemical reaction of starch and iodine (discovered independently by Stromeyer, and Colin and de Claubry), was used microchemically by Raspail in 1825. Four years later Raspail found that sulphuric acid gives a purple coloration to albumen in the presence of sugar. Raspail used this reaction either for the determination of albumen or sugar, when it was present in large quantities, as in pollen grains. Schleiden in 1838 and von Mohl in 1840 showed that after treating the cell wall with sulphuric acid, iodine caused a blue coloration of the cellulose. Ten years later, in 1850, this method was largely supplanted by Schultze's zinc chloride solution. In the same year Millon devised the test for proteins that bears his name. Thus we find that by 1850 the microchemical determination of the constituents of the plant cell was in a fairly satisfactory state. Although Schleiden gave a list of chemicals for the study of the cell in 1842, the first serviceable collection of microchemical methods is that of Schact in 1852, in which the means of recognizing many different plant products is given. Thus cellulose and xylogen can be differentiated by the reagents given, protein compounds recognized by their behavior towards iodine, nitric acid (a reaction which was pointed out by Glauber in 1686), hydrochloric acid, and Raspail's test. Starch is recognized by iodine, gums and dextrines by digestion and the formation of a flocculent precipitate in alcohol. No very sure method is given for sugar or fats, Raspail's test showing sugar when present in abundance, while fats are determined by their high refractive power and disappearance under the microscope

in the presence of alkalis. Other important advances were the application of Troemmer's reagent to microchemical analysis by Sachs in 1859 and the use of alcanna tincture by Mueller for the determination of fats. The growth of microchemistry may be seen by comparing Poulson, Behrens, Zimmermann, and Richter.

The work of the Hofmeisterian epoch which led to the foundation of our knowledge of the alternation of generations was carried out almost wholly on fresh material and with free-hand sections. Botanical study in Germany between 1840 and 1875 was dominated by von Mohl, Schleiden, Hofmeister and Naegeli, all of whom favored the use of as simple a technique as possible, their mastery of free-hand use of the razor being traditional even to the present day. It may be that the great skill which they possessed with the razor made them loath to use the microtome and although Unger and von Mohl mention the microtome they did not recommend it but gave the impression that it was an instrument for the dilletante rather than for the serious worker. Even today this idea lingers in certain quarters and is expressed in the saying that "a steady hand is the best microtome." In certain cases special methods of holding the material were recommended, von Mohl using pieces of pith for holding thin leaves and the like, whereas Unger used cork. Schleiden in 1842 described a method of fastening material to the thumb-nail with saliva or oil and then rocking the razor blade back and forth after the manner of a rocking horse. The first attempt at embedding material appears at this time. Unger tells us that Fenzl devised a crude embedding process for sectioning seeds by dropping the material to be sectioned in a hole made by a hot needle in a piece of stearine, and then the whole mass was cooled and cut. Griffith and Henfry modified this process by substituting white wax as the embedding medium. Schleiden devised another embedding method of immersing minute objects in a thick mucilage of gum arabic which was dried on a small board until glassy. After sectioning the sections were placed in water to swell them to their normal size. Staining was not used in the microtechnique, if we exclude the use of iodine. The article entitled "ovules," an English description of German methods by Griffith and Henfry, gives a good idea of the free-hand methods in use at that time. Their directions

are as follows, "The ordinary plan is to place an ovule between the thumb and fore-finger of the left hand, and with a very sharp razor cut it into two unequal parts, in the direction of the axis. The larger piece is then laid on its flat side on the finger (by the aid of a mounted needle) and another section made so as to leave a section preserving all of the central part of the ovule. This adheres either to the finger or to the razor and a drop of water should be placed on it to free it; then it may be transferred to the slide by a very fine camel's hair pencil. Examined under a low power it will probably be found to require further dissection, with exceedingly fine needles, under a simple lens, sometimes mere pressure is of service. We have found ovules which have been kept in spirits easier to dissect; when fresh, the cell membranes are excessively delicate."

The compressorium, an instrument much used by the animal histologists of that time, proved of but little service in botany, while maceration methods were used to a considerable extent for the dissociation of tissues. The earliest process, introduced by Moldenhawer, was the decaying of the wood in a manner very similar to Hill's method. Various strong acids and alkalis were also used for this purpose, the recommendations of Schleiden (1842) being typical, but after the discovery of Schulze's maceration methods little else was used. The first fluid Schulze proposed was a mixture of nitric acid and phosphoric acid but the more recent combination of nitric acid and potassium chlorate has found widespread favor among botanists. Chromic acid was also used for this purpose to some extent, having been introduced by Sanio in 1863.

The mounting media developed by the English microscopists, especially glycerine and glycerine jelly, were widely used by the German botanists of the sixties. Mounting media which were originated by botanists in the latter part of the period under discussion include the use of potassium acetate by Sanio the use of potassium hydroxide by Schulze and Hanstein's potassium hydroxide solution. The methods which were used by the German botanists at that time showed, however, no great advance and between 1860 and 1875 the progress which they made in methods is not at all comparable to the progress which the zoologists were making.

## The Development of the Modern Microtechnique

To properly understand the development of botanical microtechnique it is necessary to review the progress that the zoologists had been making in microtechnique. They soon recognized the value of Clarke's process of clearing in turpentine before mounting in balsam and various improvements were made in the procedure. As a result we find Kutschin substituting creosote for the turpentine, and two years later Rindfleisch cleared his preparations in clove oil before mounting in balsam. In the following year, 1866, Steida made an extensive study of the clearing action of about 30 essential oils, and among others recommended bergamot oil.

Although attempts at staining were first made by microscopists on botanical material, or by botanists, the development of the technique of staining is almost wholly due to the zoologists. I have been unable to find anywhere recognition of John Hill as the real pioneer in the history of staining. Another process, which is essentially similar to staining, is that of Reade who charred plant tissues in order to render them more visible when mounted in balsam. Queckett in 1848 recommended charring or staining with either iodine, fustic or logwood extracts to bring out the structure of plant tissues mounted in balsam. In 1843 Goppert and Cohn used carmine for the study of the cell contents of Nitella. A few years later Hartig (1854), used carmine for staining the cell contents and noted that the nucleus was not stained until after the death of the cell. Osborn also used carmine for his studies on the root-tip of the wheat plant. The data on the history of staining have been collected by Gierke and later by Apáthy. Gierke compares the claims of Goppert and Cohn, Hartig, and Osborn and thinks that Hartig should be given the credit for discovering the process of staining tissues with carmine. Apáthy thinks that the real credit for the discovery belongs to Corti, a work which Gierke apparently overlooked. In my opinion, for reasons given above, Hill should be credited with having first used staining methods in connection with microscopical work. Queckett's work also antedates that of Corti or Hartig, although Corti should retain the honor of being the first to apply staining methods to the study of the contents of the cell. Previous to the study of the early literature, Gerlach was generally

credited with having first used stains. Although this has been shown to be false, Gerlach's use of stains is important for it was through the adoption of his methods that staining came into general use. Apáthy has made a very apt comparison when he says that Corti and Hartig were the Normans (Norsemen?) of staining while Gerlach was the Columbus.

Experimentation also began on the value of other substances for staining tissues. Waldeyer is held by both Gierke and Apáthy as the first to use hæmatoxylin as a stain. This is incorrect, since Queckett (1848) and Wigand had both used logwood stains before Waldeyer. In 1862 Wigand made an extensive study of the behavior of plant tissues towards coloring matters. This work was a study of the phenomena connected with the staining of plant tissues from the standpoint of the commercial dyer rather than from that of the microscopist trying to bring out structural differences. Besides cochineal he stained with hæmatoxylin and certain other colored plant extracts, as yellow wood (old fustic), cutch, and root extracts. Negative results were obtained with indigo and various mineral stains. A selective staining of different parts of the plant was noted but no use was made of this except to help elucidate the theory of staining that he proposed. The first hæmatoxylin stain of real value was that of Boehmer, who, in 1865 gave a formula that is in use to the present day.

Soon after the discovery of aniline dyes, we find the application of them to the staining of microscopical preparations. The first to use them was Benecke, although at the present day we do not know what his "Lila-Anilin-Farbe" represents. In the next year (1863) Waldeyer employed Rosaniline (Aniline Red), Anilien (Aniline Violet), and Parisian Blue (Aniline Blue). In the same year there was the independent discovery of the staining power of Magenta (the English name for Fuchsine) by Lynde and by Roberts. Lynde used Magenta for staining the contents of plant cells and noted that the contents were not stained until after the death of the cell, while Roberts stained blood corpuscles with Magenta. In the following year Abbey experimented with a still larger number of dyes. In studying the contents of the plant cell he used Mauve, Hoffmann's Green (Iodine Green), Aniline Brown, Picrate of Aniline, Magenta, Aniline Green and two blue colors the names

of which are not given. Except for the work of Lynde and Abbey practically all of the early work on staining with aniline dyes was done by zoologists. The list of discoveries along this line might be prolonged indefinitely; for the first twenty years following the introduction of staining with aniline dyes by Benecke, Gierke cites 55 references, each of which contains something that constitutes a distinct advance over methods known up to that time, and all but three refer to zoological or medical publications. Among the stains most used by botanists today may be noted the introduction of Dahlia by Huguenin in 1874, Eosin by Fischer in 1875, Methyl Violet, Iodine Violet and Safrain by Ehrlich in 1877, Bismarck Brown by Weigert in 1878, and Methylene Blue by Ehrlich in 1881. It must be remembered that these stains were used singly and not in combination as we now use them. The first double staining is that of Schwarz, who in 1867 stained his material in carmine and then in picric acid. Other early combinations of stains were Eosine and Methyl Green, Eosine and Dahlia, and Eosin and Methyl Violet. The method now most generally used, that of overstaining in a solution of the dye and then destaining to the proper intensity became well known through the work of Flemming (1881), although the process had been previously used by Bœttcher and Hermann. Flemming experimented with a large number of dyes to find which one gave the best nuclear stain, among those used were Safrainin, Magdala Red, Dahlia, Mauve, Rouge Fluorescent, Solid Green, Ponceau, Fuchsine, Eosine, and Bismarck Brown. Later Flemming (1884) found another good stain in Gentian Violet. Safranin and Gentian Violet as a double nuclear stain was not suggested by Flemming as is frequently stated, but was first employed by Brazzola; while the much used triple stain of Safranin, Gentian Violet, and Orange G was combined by Flemming in 1891.

The development of embedding methods is also due to zoologists. The term embedding should be used with care since it has been used by investigators in two different senses. In one case there is merely the surrounding of the material with any medium, the Fenzl method mentioned above being an example. In the other embedding process the tissue is completely saturated with

the embedding medium. The first process might well be called an enclosing method, while Apáthy has suggested the term interstitial embedding for the latter. Paraffine was introduced into microtechnique, as an enclosing medium, by Klebs in 1869. Other substances used for the enclosing method of embedding were gum arabic by Heidenhain, glycerine jelly by Klebs, and albumen by Bresgen and Calberla. In the enclosing methods of Strickler and of Born the material was dehydrated, cleared with an essential oil and then placed in a mixture of wax and oil, while after sectioning some solvent was used to remove the enclosing medium.

Although Schleiden's method introduced in 1842 was an interstitial embedding process the first serviceable method is that of Flemming, who in 1873 used transparent soap. Bergamot oil was used as the solvent for the paraffine in the older embedding methods, but it has since been shown that paraffine does not dissolve in bergamot oil to any appreciable extent. Turpentine as a solvent for the paraffine was not generally adopted since it caused plasmolysis. The independent discovery of cholorform as the solvent for the paraffine by Griesbrecht and Bütschli, in 1881, brought the method up to a point where there could be an interstitial embedding of the most delicate tissues without plasmolysis. The other embedding medium which is widely used today, celloidin, was introduced by Duval in 1879. Duval used collodion, but its use was abandoned after Schiefferdecker showed the greater adaptability of the patent collodion called celloidin.

Fixing solutions are a comparatively recent development. The older investigators were more anxious to obtain some substance that hardened the tissue than to obtain what we now call fixation. The different editions of Lee reflect well this change in attitude towards the hardening agents. Chromic acid was one of the earliest hardening agents introduced, Hannover using it in 1840. The term fixation came into use in the early eighties and practically all of our fixing mixtures were proposed in the decade of 1880-1890. Lang used mercuric bichloride as a fixing medium either alone, or in combination with acetic acid, picric acid, or alum. Osmic acid, although introduced into microtechnique by Schultze in 1864 was not used as a fixing medium until Flesch combined it with chromic

acid. Flemming experimented with a number of combinations containing osmic acid and decided that a mixture of osmic, acetic, and chromic acids gave the best result. The first formula published is now called the "weak" formula since two years later in 1884, he gave another mixture of the same ingredients in greater concentration, forming what is called the "strong" mixture. These few citations are not given in an attempt to cover the field but more in an endeavor to show the period in which methods of fixation came into general use.

The general opinion seems to be that the microtome is of fairly recent origin. Thomé states that the greater number of microtomes go back to two fundamental types, the Ranvier and the Rivet microtome. Minot thinks that the first microtome which resembles the modern microtome is the instrument of His, the Valentine double-

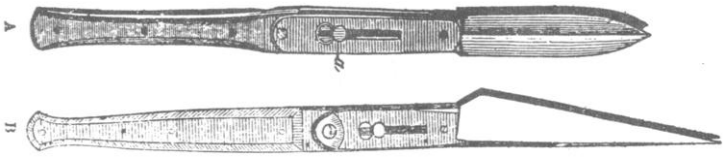


Fig. 14.—Valentine's double-bladed knife. (Queckett, 1848).

bladed knife being regarded as a forerunner of the modern microtome. In discussing the development of microtechnique before 1800 I have mentioned the microtomes of Adams, Custace, and Cummings, while it was also shown that the English microscopists used similar instruments more or less commonly in the years succeeding 1800. Valentine's double-bladed knife (Fig. 14), invented in 1839, has been of limited service in animal histology, and is even sold to the present day. Another instrument, called a micro-

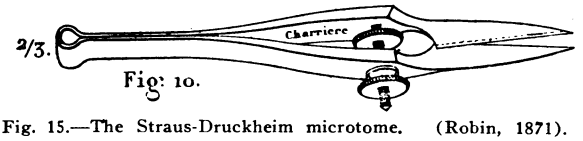



Fig. 15.—The Straus-Druckheim microtome. (Robin, 1871).

tome, is that of Straus-Druckheim. This, as is shown in Figure 15, is really a pair of dissecting scissors, the blades of which are prevented from cutting their full length by means of a screw.

In 1843 Oschatz invented an instrument for cutting sections which he called a microtome. This term was generally accepted by German histologists but the English microscopists used the term cutting machine or cutting engine, reserving the name microtome for the Straus-Druckheim instrument. Robin has protested against calling sectioning instruments microtomes maintaining that the term should be only employed in connection with the Straus-Druckheim instrument. This is not justified on the grounds of priority since in 1839 Chevalier called the sectioning instruments of Cummings and Adams, "le *couteau micrometrique* ou mieux l' *instrument*



Fig. 16.—Ranvier's microtome. (Dipple, 1882).

*microtomique.*" From this we may judge that the coiner of the word microtome (Chevalier) used it to describe any instrument for cutting sections.

The mechanical principle of Cummings and Adams microtomes is found, with one exception, in all of the microtomes made before 1868. Although varying considerably in detail all of these microtomes have a holder in which the material is clamped and then raised through a cylinder by means of a screw. The Oschatz microtome was made in two forms, either a simple hand microtome or a table form that had a three legged base. Welcker's microtome was very similar to that of Oschatz. Other microtomes which were made in the middle of the last century were those of Smith in 1859, Schmidt in 1859, and Luys in 1868. The microtomes of Nachet and Collin appeared some time before 1870 but I have been unable

to find the exact date. Other microtomes were also made judging from Harting's statement (in 1864) that there was a very old microtome in the Utrecht Cabinet, while Unger mentions using a cutting machine made by Plossl of Vienna. The microtome of Ranvier (Fig. 16) presents no further advancement in the construction of the instrument, in fact being one of the simplest ever devised, but it was through the use of the instrument by so eminent an investigator that the microtome came into real favor among zoologists. Gudden improved the Ranvier microtome by fastening it to the table, but the fastening of the microtome to the table had been described years before by Topping and by Pritchard and was well known to all the English microscopists of the fifties.

Another mechanical principle used in the microtome is the gradual raising of the object holder by pushing it along an inclined plane. The first application of this principle is generally described to Rivet, but Capanema had made use of the principle twenty years before the invention of Rivet's microtome. Harting is the only one who recognized the value of this instrument and he stated that it was the best microtome which had been made up to that time. Harting reproduces two of the five figures from Capanema's plate but the vertical section is shown upside down so that at a cursory glance there would appear to be little of value in the machine. The instrument was only about 2½ inches long, Fig. 17A, showing a trans-

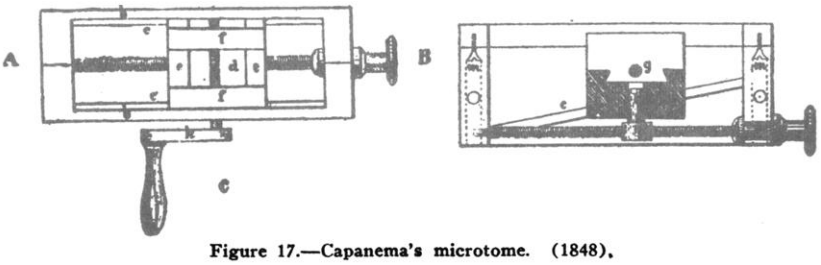



Figure 17.—Capanema's microtome. (1848).

verse cross section, the chief point of interest being the object holder. This consisted of two clamps (ff) which were regulated by a screw (g), a plate (d) forming the base of the holder. After the material had been tightened in place by a crank (k), the crank was removed from the screw. The object holder slid on two lateral

inclined planes (cc in Fig. 17B) and from its bottom a pillar (1) extended downwards, the turning of another screw (x) moving the object holder. As the screw (x) turned it caused the holder to move up the inclined plane and this slowly raised the material held in the clamp. The sections were cut by means of a knife that slid along two guide plates (bb).

Rivet's microtome (Fig. 18) is the fundamental type on which all improvement of the sliding microtome have been made. Botanists should be interested in this microtome since it was devised for cutting plant tissues. In the advertisements inserted in the 2nd. edition of Nægeli and Schwendener we find Rivet's microtome ad-

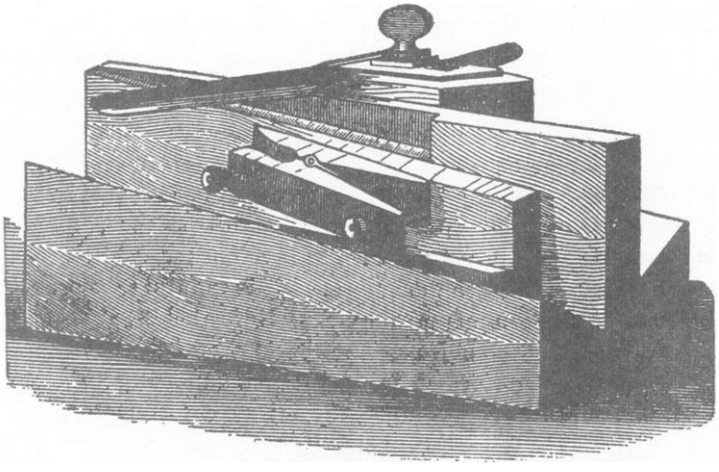


Fig. 18.—Rivet's microtome. (Grönland, 1878).

vertised for cutting plant material, while Ranvier's microtome is listed for making animal sections. The microtome as invented by Rivet was first made entirely of wood by Verick of Paris in 1868. Minot states that it was described in the Annales des Science Naturelles but the first description that I have been able to find is that of Grönland. The microtome consisted of three parts a central block and a separate carrier for the material and the knife. The central block had a base measurement of 16 by 6 cm, and a height of 6 cm. On either side of this block there were wedge shaped grooves so that the middle upper portion was only 13 mm. in thick-

ness. The groove at the left had a slope of 1:100, whereas the right hand groove was parallel to the top of the instrument. On the top of the middle portion there was a scale parallel to the sloping left hand groove, and this was divided so that each division corresponded to a vertical elevation of 1:100 of a millimeter. A block that was fitted in the right hand groove carried the knife and another in the left hand sloping groove carried the object holder. The apparatus for holding the material was very simple, being fashioned after a patent American clothes-pin. This clothes-pin holder was fastened to the block so that no orientation of the material was possible. The microtome was operated by drawing the knife towards the operator and then shoving ahead the block in the inclined groove the desired distance, the height which the block was raised being computed from the scale at the top of the microtome. After drawing the knife forward the process was repeated.

Brandt constructed the sliding microtome of metal instead of wood, and since this instrument was made by Leyser it is often called the Rivet-Leyser microtome. Grönland considers this substitution of metal for wood a step backwards. A still more important improvement in the microtome is the introduction of the mechanical advancing of the object holder by means of the screw, as found in the Schanz microtome. The other essential improvement was the discarding of the primitive clothes-pin type of object holder and the substitution of a holder capable of being rotated in any direction. Further improvements in this type of microtome have been chiefly variations in already existing principles used in its construction. Perhaps the most notable of these has been the introduction of an automatic device for raising the holder and a mechanical means of operating the knife carriage.

The Caldwell and the Rocking microtome, both types of automatic instruments, were introduced by the Cambridge Scientific Instrument Company in 1885. The Caldwell automatic microtome is of historical interest only, since it was used but little, but in its simplified form, as the Cambridge rocking microtome, it gained a widespread popularity. Since this instrument is so generally known no description of it is necessary. The mechanical error of cutting sections in a curved plate instead of a flat plate has been overcome

in later models. Ryder's microtome was invented in 1888 but did not come into general use since the mechanical principle underlying the construction of this instrument was also incorrect because the paraffine block did not pass evenly across the edge of the knife but moved in the arc of a very short circle. In spite of this defect one enthusiastic reviewer said that this was "undoubtedly the microtome of the future."

Microtomes of the rotary type are probably the most generally used at the present time. They are an American invention, the idea having been worked out independently by Pfeifer, a mechanican at Johns Hopkins University, and by Minot of Harvard. The first published description is that of the so-called "Johns Hopkins" microtome in 1886. Only one or two instruments of this type were ever manufactured, but it is interesting to note that one of them is still in active service today in the laboratory at John Hopkins University. Minot's microtome was made in 1887 by Baltzer of Leipzig, although the manufacture was soon transferred to Zimmermann, who still makes them. The first published description of these microtomes appeared in 1888.

In discussing the development of the modern botanical microtechnique the problem is largely one of finding out at what particular time methods devised by animal histologists were first applied to botanical problems. To really appreciate how deeply indebted we are to the zoologists it is only necessary to read the names of the fixing solutions and stains used in publications in any botanical periodical. The almost exclusive appearance of the names of Flemming, Heidenhain, Merkel, Hermann, Pianaese, and many other animal histologists in connection with descriptions of methods of study shows this very well.

Mon took 1870 as the date from which he commenced his discussion of the modern botanical microtechnique, whereas Strasburger took 1875 as the starting point for a review of the modern cell theory; but since there was comparatively little development of microtechnique in the decade of 1870-1880, it matters little which date is taken. The article of Strasburger, published in 1875, may be taken as representative of the most progressive methods in use at that time. In the study of cell contents no staining methods were

used but the necessity of arresting the progress of nuclear division, in order to allow time for a more detained study, was recognized, and absolute alcohol was used as the fixing medium. Permanent preparations were made by transferring the material fixed in alcohol to glycerine. Whenever the material was plasmolyzed by the strong alcohol, a more dilute solution was used for fixation. In some cases iodine was employed as a stain.

That the methods which were being developed at this time by the zoologists were not entirely neglected by the botanists as is shown by the fact that every new method of importance was reviewed in Just's Jahresbericht. Between 1875 and 1880 the most noticable progress in botanical microtechnique was the gradual adoption of staining methods. Among the pioneers may be mentioned Errera who stained nuclei with Nigrosine, Strasburger who used Methyl Green and acetic acid for simultaneous fixation and staining, and the use of Methyl Green by Treub. All of this staining of the cell contents was with a single stain only.

As shown in the discussion of the history of staining most of the very early work with botanical material was to bring out the cell walls. In the same way the first double staining of plant tissues was for the demonstration of fibro-vascular bundles and not for the structure of the cell contents. The development of double staining methods for stems and other tissues was due to the desire of microscopists to make striking microscopical preparations rather than to bring out morphological structures. Some of the earliest work was done by the American microscopists in the late seventies. Among the combinations used, hæmatoxylin and Aniline Blue by Poole, Crawshaw's Aniline Blue and Magenta by Barrett, Carmine and Aniline Green by Peet, while Rothrock used hæmatoxylin or carmine in combination with Iodine Green. Richardson made the most extensive series of experiments on staining among others a triple stain of Atlas Scarlet, Soluble Blue and Iodine Green. The first double staining for the demonstration of cell contents is McFarlane's combination of Diamond Fuchsine and Methyl Green.

During the late seventies other methods used by zoologists were introduced into botanical microtechnique. Leitgeb cleared preparations with clove oil before mounting in 1875; while Parker

in fixed Chara in a mixture of chromic and osmic acid and after dehydration in alcohol cleared in clove oil and imbedded in cocoa butter.

The best index to the expansion of modern botanical microtechnique is Strasburger's "Botanische Prakticum"; the different editions of which cover a period from 1884 to 1913. At the time that the first edition appeared we find the modern methods of study fairly well begun. The preëminent fixing fluid recommended is absolute alcohol. It is true that chromic acid, concentrated picric acid, and mixtures of chrome-acetic and osmic-chrome-acetic acids are cited in connection with the study of the algæ but since alcohol is the only medium recommended for the study of the anther, ovary, and meristematic tissue the technique of fixation may be regarded as quite primitive at that time. With the exception of MacFarlane's Diamond Fuchsine-Methyl Green mixture, single stains only were used for the study of the cell contents. Carmines and hæmatoxylins appear most frequently in the pages of the first edition although the regressive staining with aniline dyes, so strongly recommended by Flemming in the early eighties, is described in connection with the studies on nuclear division, Safranin and Gentian Violet being considered the best. Double staining is emphasized in connection with the work on vascular bundles and three different processes are described, the use of Grenacher's Alum Carmine-Methyl Green Picro-Nigrosine and Picro-Aniline Blue.

The process of interstitial embedding in paraffine, celloidin, or soap was well known to the zoologists when the first edition appeared and a description of all of these methods is given but no direct application to the study of plant tissues can be found in the book. Strasburger refers to Koch (1874) as having used paraffine embedding in the study of plant tissues, but this method was one of enclosing rather than interstitial embedding.

The chief advance as recorded in the second edition is the use of a much larger number of stains for the study of the plant cell. There is also a much more complete discussion of the process of embedding, but, with the exception of a note on the arranging of serial cellodin sections of anthers there is again nothing said about the application of embedding methods in botanical work.

To Francotte belongs the credit of introducing the paraffine method into botany. It is true that the notes on the methods for the study of leaves, stems, anthers, ovaries, and fungi cover but three pages but this is the first case where complete schedules are given for the different processes leading to the embedding of plant tissues in paraffine. Francotte did not think that paraffine was the best medium for stems and leaves, but gave the preference to soap, with cellodin as the alternative. Apparently this work has been entirely overlooked, since neither Schönland, Moll, nor Koch mention it. The years 1887-1892 mark the establishment of the paraffine. Attention was called to the usefulness of paraffine through the publication of Schönland and Moll appearing in the year 1887. The first method Schönland used was the gradual dehydration with methyl alcohol and then a transfer to clove oil and from the clove oil to oil of turpentine; afterwards the tissues were placed in paraffine with a melting point of 45 degrees Centigrade. Later (1888) he substituted ethyl for methyl alcohol and also interpolated a further step by allowing the material to remain in turpentine saturated with paraffine before the transfer to pure paraffine. Moll's method differs very little from that which we now use, the chief divergence from the present schedules lies in a much slower dehydration, several hours being allowed for each grade of alcohol into which the material was placed. Campbell and Koch helped to propagate the doctrine of embedding, by showing its applicability to large numbers of plants. With the appearance of Koch's work we may consider the availibility of the paraffine method for botanical microtechnique as well established, although many articles published at that time continued to give full directions for the process.

Historically the soap method is older than that of paraffine, but in their introduction into botanical microtechnique the two are coincident. Pfitzner's description of the soap method in connection with plant tissues appeared the same year as Schönland's and Moll's articles, but the greater adaptability of paraffine has prevented the widespread use of soap although Wilcox and Osterhout are advocates of its use in special cases. The first complete description of cellodin embedding for plant tissues is that of Busse. This substance has always been used to a large extent in section-

ing woods the most generally used method at the present day being the so-called "Harvard Method" described by Plowman.

The dominance of the school of cytologists led by Strasburger has been most strongly felt in Germany, England, and America. The disciples of Strasburger have generally advocated the mastery of a few methods and after the time of his publication of the process of embedding in paraffine and staining with Flemming's triple stain (1896) this has come to be regarded as the universal method in certain quarters. In other centers, notably the French botanists under the leadership of Mangin and Guignard, there has been the use of a much wider range of stains. At present it may be said that there is a general drift towards a more varied attack. This is perhaps due to the increasing interest in the cytology of the Cryptogams, in which there is a less uniform behavior towards the tripple stain of Flemming than there is in the Spermatophytes. This has led to the use of Pianæsæ's stain, Azur Blue, and several others. On the other hand the investigation of cellular organization as exemplified in the study of mitochondria, has called for the development of a special staining technique. With the opening up of these new fields we may confidently look forward to further developments along this same line.

Methods of fixation have not multiplied as rapidly as the methods of staining. A few new fixing fluids have been proposed by botanists, that of Juel being an example; while the French and Belgian botanists have taken several mixtures devised by zoologists and recast the formulæ so that the fluids can be used for the study of plant tissues. On the other hand there is also a strong tendency toward a critical examination of the action of fluids in general use at the present time, the work of Fischer and Lundegard being typical examples.

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